

Appl. No. 10/026,140  
Amdt. dated March 5, 2004  
Reply to Office action of October 7, 2003

Page 3

**IN THE CLAIMS:**

The claims as currently presented and under consideration, are presented below for the Examiner's convenience and to comply with 37 CFR §1.121. This listing of claims will replace all prior versions, and listings, of claims in the application:

1. [Currently Amended] ~~An isolated~~ polynucleotide isolated derived from a fungal source, which polynucleotide comprises a nucleotide sequence encoding a  $\beta$ -glucosidase 5 ~~an enzyme~~ having  $\beta$ -glucosidase activity.
2. [Currently Amended] An isolated polynucleotide derived from a fungal source, which polynucleotide comprises a nucleotide sequence encoding an enzyme having  $\beta$ -glucosidase activity selected from the group consisting of:
  - (a) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a  $\beta$ -glucosidase 5 BGL5 polypeptide having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
  - (b) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a  $\beta$ -glucosidase 5 BGL5 polypeptide having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
  - (c) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL5 polypeptide having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
  - (d) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL5 polypeptide having the amino acid sequence presented in Figure 2;
  - ~~(e) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL5 polypeptide having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;~~
  - ~~(f) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL5 polypeptide having the amino acid sequence presented as SEQ ID NO:2;~~

Appl. No. 10/026,140  
Amdt. dated March 5, 2004  
Reply to Office action of October 7, 2003

Page 4

(eg) a nucleic acid sequence presented as SEQ ID NO:3, or the complement thereof; and

(fh) a nucleic acid sequence that hybridizes, under high stringency conditions to the sequence presented as SEQ ID NO:3, or the complement or a fragment thereof, wherein said isolated polynucleotide encodes a polypeptide having the biological activity of a  $\beta$ -glucosidase.

3. [Original] The isolated polynucleotide of Claim 2, wherein % identity is calculated using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix.

4. [Original] The isolated polynucleotide of Claim 2, wherein hybridization is conducted at 42°C in 50% formamide, 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100  $\mu$ g/ml denatured carrier DNA followed by washing two times in 2X SSPE and 0.5% SDS at room temperature and two additional times in 0.1 SSPE and 0.5% SDS at 42°C.

5. [Original] The isolated polynucleotide of Claim 2, wherein said polynucleotide is an RNA molecule.

6. [Currently Amended] The isolated polynucleotide of claim 1 encoding an enzyme having  $\beta$ -glucosidase activity, wherein the enzyme is derived from a ~~Trichoderma~~ *Trichoderma* source.

7. [Currently Amended] The isolated polynucleotide of Claim 6, wherein the enzyme is derived from ~~Trichoderma reesei~~ *Trichoderma reesei*.

8. [Currently Amended] An expression construct ~~including~~ comprising a polynucleotide sequence (i) having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2), or (ii) being capable of hybridizing to a probe designed to ~~derived from~~ the nucleotide sequence encoding the amino acid sequence disclosed in Figure 2 under conditions of intermediate to high stringency, or (iii) being complementary to a nucleotide sequence encoding the amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2).

Appl. No. 10/026,140  
Amdt. dated March 5, 2004  
Reply to Office action of October 7, 2003

Page 5

9. [Currently Amended] A vector ~~including~~ comprising the expression construct of Claim 8.
10. [Original] A vector comprising an isolated polynucleotide of Claim 2, operably linked to control sequences recognized by a host cell transformed with the vector.
11. [Original] A host cell transformed with the vector of Claim 9.
12. [Original] A host cell transformed with the vector of Claim 10.
13. [Original] The host cell of Claim 12, which is a prokaryotic cell.
14. [Original] The host cell of Claim 12, which is a eukaryotic cell.
15. [Original] A recombinant host cell comprising a polynucleotide of Claim 2.
16. [Original] The recombinant host cell of Claim 15, which is a prokaryotic cell.
17. [Original] The recombinant host cell of Claim 15, which is a eukaryotic cell.
18. [Cancelled]
19. [Original] A method of producing an enzyme having  $\beta$ -glucosidase activity, comprising:
- (a) stably transforming a host cell with an expression vector comprising a polynucleotide as defined in Claim 2;
  - (b) cultivating said transformed host cell under condition suitable for said host cell to produce said  $\beta$ -glucosidase; and
  - (c) recovering said  $\beta$ -glucosidase.
20. [Original] The method of Claim 19 wherein the host cell is a filamentous fungi or yeast cell.
21. [Cancelled]
22. [Currently Amended] A recombinant host cell comprising a deletion or insertion or other alteration in the *bgI5* gene which inactivates the gene and prevents beta-glucosidase 5 BGL5 polypeptide production.
23. [Currently Amended] An antisense oligonucleotide complementary to a messenger RNA that encodes a beta-glucosidase 5 BGL5 polypeptide having the sequence presented as SEQ ID NO:2, wherein upon exposure to a  $\beta$ -glucosidase-producing host cell, said oligonucleotide decreases or inhibits the production of  $\beta$ -glucosidase by said host cell compared to a control host cell.
24. [Original] The antisense oligonucleotide of Claim 23, wherein the host cell is a

Appl. No. 10/026,140  
Amdt. dated March 5, 2004  
Reply to Office action of October 7, 2003

Page 6

filamentous fungi.

25. [Cancelled]

26. [Currently Amended] A method of expressing a heterologous polypeptide having  $\beta$ -glucosidase activity in an ~~Aspergillus~~ *Aspergillus* species, comprising:

- (a) Providing a host ~~Aspergillus~~ *Aspergillus* with an expression vector comprising a polynucleotide encoding a signal sequence linked to a polynucleotide encoding a heterologous  $\beta$ -glucosidase according to claim 2, thereby encoding a chimeric polypeptide;
- (b) Cultivating said host ~~Aspergillus~~ *Aspergillus* under conditions suitable for said ~~Aspergillus~~ *Aspergillus* to produce said chimeric polypeptide, wherein said chimeric polypeptide is produced.

27. [Cancelled]

28. [Cancelled]

29. [Cancelled]

30. [Cancelled]

31. [Cancelled]

32. [Cancelled]

33. [Cancelled]

34. [Cancelled]

35. [Cancelled]

36. [Cancelled]

37. [Cancelled]